CLAIMS

1. A method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, said method comprises the steps of:

performing a nucleic acid amplification reaction of the target nucleic acid using a test solution containing a primer, substrate molecule at least one of which is labeled with a marker molecule capable of generating a detectable signal, a nucleic acid synthase, and a target nucleic acid molecule;

measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

evaluating mobility of the labeled molecule in the test solution on the basis of the signal detected; and

quantifying the target nucleic acid molecule on the basis of evaluation results.

2. A method according to claim 1, wherein the measurement step includes a step of measuring an amount of the marker molecule present in a predetermined measurement area.

3. A method according to claim 2, wherein, in the measurement step, a moving amount of the marker molecule within a predetermined time interval is measured for a plurality of times.

4. A method according to claim 3, wherein the evaluation step includes a step of converting a change

of the moving amount into a statistical data, on the basis of a plurality of measurement data.

5. A method according to claim 4, wherein the conversion step includes a step of performing a arithmetic operation by means of an autocorrelation function.

6. A method according to any one of claims 1 to 5, wherein,

the method further comprises a step of removing the labeled substrate molecule not incorporated into the nucleic acid amplification products, between the step of performing the amplification reaction and the measurement step; and

the measurement step is performed in the state noncontact with the test solution.

7. A method according to any one of claims 1 to 5, wherein the quantification step include a step of determining the presence and absence of the marker molecule incorporated into the amplification products during the nucleic acid amplification, on the basis of the evaluation results.

8. A method according to any one of claims 1 to 5, wherein the quantification step includes a step of determining a number of the labeled nucleic acid molecules incorporated into the amplification products during the nucleic acid amplification on the basis of the evaluation results.

9. An apparatus of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, said apparatus comprises:

holding means for holding a test solution containing a primer, substrate molecules at least one of which is labeled with a marker molecule capable of generating a detectable signal, a nucleic acid synthase, and a target nucleic acid;

measuring means for measuring a signal from the marker molecule after initiation of a nucleic acid amplification reaction in the test solution;

evaluation means for evaluating mobility of the marker molecule in the test solution on the basis of the signal detected; and

data output means for outputting a evaluation result obtained by the evaluation means as a quantification data of the target nucleic acid molecule.

10. An apparatus according to claim 9, wherein the measuring means comprises an optical system for performing measurement in a micro detection field brought within a diffraction-limited region near a focal point.

11. An apparatus according to claim 9, wherein the measuring means comprises a microscope for performing measurement in a micro filed of vision formed by a confocal optical system.

12. An apparatus according to claim 10 or 11,

wherein the diffraction-limited region is formed of an aperture having an average diameter of 30 ± 20 μm.

13. An apparatus according to claim 10 or 11, wherein the diffraction-limited region is formed of an aperture having an average diameter of 20 ± 10 μm.

14. An apparatus according to claim 10 or claim 11, wherein the micro detection field is a virtually a cylindrical region having an average radius of 200 ± 50 nm and an average length on an optical axis of 2000 ± 500 nm.

15. An apparatus according to claim 9, wherein the evaluation means comprises a means for storing a plurality of measurement data obtained in a predetermined time, and an arithmetically operating means for processing the plurality of measurement data stored in accordance with an autocorrelation function.

16. An apparatus according to claim 15, wherein the evaluation means comprises a means for storing measurement data regarding to a plurality of marker molecules obtained in a measurement area, and an arithmetic operating means for processing the measurement data stored per each of the marker molecules in accordance with the autocorrelation function.

17. An apparatus according to claim 15 or 16, wherein the data output means includes a conversion means for converting the measurement data into statistical data expressing a positional change with

time with respect to a plurality of monitoring data.

18. A method of quantitatively analyzing a target nucleic acid molecule present in a biological sample, comprising:

an amplifying step of amplifying the target nucleic acid by using first and second primer molecules having sequences which are complementary with two discrete nucleotide sequence regions of the target nucleic acid molecule respectively, at least one of the first and second primers being labeled with a detectable marker molecule and at least the number of labeled primer molecules being known;

a measurement step of obtaining measurement data regarding the labeled molecule by using at least a part of a test solution which has been subjected at least a single amplification step; and

a determination step of determining a number and a size of the target nucleic acid molecules on the basis of the measurement data.

19. A method according to claim 18, wherein the amplification step is performed by using the first and second primers which are contained in a mix ratio selected to attain asymmetric nucleic acid amplification.

20. A method according to claim 18, wherein the number of one of the first and second primers is lower than that of the other primer, and the primer present

in a lower number is labeled with a marker molecule.

21. A method according to claim 20, wherein the mix ratio of the first and second primers in the test solution falls within a range of 2:1 to 20:1.

22. A method according to claim 20, wherein a mix concentration ratio of the first and second primers in the test solution falls within a range of 800 nM :400 nM to 800 nM :40 nM.

23. A method according to claim 18, wherein the measurement step includes

a step of obtaining a plurality of measurement data within a predetermined time interval, in a micro detection field capable of identifying individual marker molecules;

a step of converting the plurality of measurement data into a statistical data showing a positional change with time; and

a step of determining a number of target nucleic acid molecules on the basis of the statistical data.

24. A method according to claim 23, further comprising a step of performing arithmetic operation of the statistical data by use of autocorrelation function.

25. A method according to claim 24, wherein a fluctuation motion of the marker molecule in the test solution is measured in the measurement step.

26. A method according to any one of claims 18 to 25, wherein the determination step is performed on the

basis of a curve which is obtained by plotting the statistical data and shows a dynamic change of the target molecule.

27. A method according to claim 23, wherein the measurement step is performed in a three dimensional micro detection field.

28. A method according to claim 27, wherein the micro detection field in the measurement step is formed by a confocal optical system.

29. A method according to claim 28, wherein the micro detection field is a diffraction-limited region near a focal point.

30. A method according to claim 29, wherein the diffraction-limited region is formed by a pin hole having an average diameter of 30 ± 20 μm.

31. A method according to claim 29, wherein the diffraction-limited region is formed by a pin hole having an average diameter of 20 ± 10 μm.

32. A method according to any one of claims ~~26~~ 27 to 31, wherein the micro detection field is virtually a cylindrical region having an average radius of 200 ± 50 nm and an average length on an optical axis of 2000 ± 500 nm.

33. A method according to claim 18, wherein the marker molecule comprises a fluorescent dye.

34. A method according to claim 33, wherein the fluorescent dye generates a detectable signal both

before and after hybridization.

35. A method according to claim 34, wherein the fluorescent dye is selected from the group consisting of FITC, DAPI, rhodamine, Cy 3, Cy 3.5, Cy 5, Cy 5.5, and Cy 7.

36. A method according to claim 32 or 33, wherein the measurement step is performed by a measurement mode for measuring a single photon.

37. A method according to claim 18, wherein the amplification step is performed in a number of cycles which is determined depending upon an amount of the target nucleic acid molecule.

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